120307514 CA: 120(24)307514f PATENT
Medicinal composition containing TCF-II
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PATENT: Can. Pat. Appl. ; CA 2100720 AA DATE: 940117
APPLICATION: CA 2100720 (930716) *JP 92212229 (920716) *JP 92212227 (920716) *JP 92234198 (920810)
PAGES: 59 pp. CODEN: CPXXEB LANGUAGE: English CLASS: A61K-037/02A; A61K-037/36B
SECTION:
CA263006 Pharmaceuticals
CA201XXX Pharmacology
IDENTIFIERS: pharmaceutical TCFII liver disease hypoproteinemia, wound healing TCFII pharmaceutical, injection pharmaceutical TCFII, ointment pharmaceutical TCFII
DESCRIPTORS:
Kidney,disease, failure... Malnutrition,undernutrition...
hypoproteinemia from, treatment of, TCF-II-contg. pharmaceutical compn. for
Pharmaceutical dosage forms,injections...
of TCF-II, for treatment of liver disease and hypoproteinemia
Pharmaceutical dosage forms,ointments...
of TCF-II, for wound healing
Glycoproteins,specific or class...
TCF-II, pharmaceutical compns. of, for treatment of liver disease and hypoproteinemia and for wound healing
Wound healing...
TCF-II-contg. pharmaceutical compn. for
Biliary tract,disease, intrahepatic cholestasis...
treatment of damage from, TCF-II-contg. pharmaceutical compn. for
Burn... Cirrhosis... Hepatitis,acute... Hepatitis,chronic... Liver,disease ... Liver,disease, fatty... Proteins,metabolic disorders, hypoproteinemia,biological studies... Skin,disease, decubitus ulcer... Skin,disease, injury...
treatment of, TCF-II-contg. pharmaceutical compn. for

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An agency of Industry Canada

CA 2100720 C 2003/03/11

(11)(21) 2 100 720

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 1993/07/16

(41) Mise à la disp. pub./Open to Public Insp.: 1994/01/17

(45) Date de délivrance/Issue Date: 2003/03/11

(30) Priorités/Priorities: 1992/07/16 (212227/1992) JP;
1992/07/16 (212229/1992) JP;
1992/08/10 (234198/1992) JP

(51) Cl.Int.^{6}/Int.Cl.^{6} A61K 38/18

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(54) Titre : COMPOSITION MEDICINALE CONTENANT DU TCF-II
(54) Title: MEDICINAL COMPOSITION COMPRISING TCF-II

(57) Abrégé/Abstract:
Medicinal compositions comprising of TCF-II derived from human fibroblast cells, are particularly effective for liver diseases treatment, protein synthesis stimulation, and wound healing.

2100720

Abstract

Medicinal Composition Comprising TCF-Ⅱ

Medicinal compositions comprising of TCF-Ⅱ derived from human fibroblast cells, are particularly effective for liver diseases treatment, protein synthesis stimulation, and wound healing.

Title of the Invention

Medicinal Composition comprising TCF-Ⅱ

Background of the Invention

This invention relates to medicinal compositions containing an effective amount of TCF-Ⅱ, more particularly for the treatment of liver diseases, stimulation of protein synthesis and healing of wounds.

Description of the Prior Art

Biologically active substances produced by human derived fibroblast cells, for example β-interferon as a tumor cytotoxic factor, have been well known.

Biologically active substances produced by fibroblast cells other than β-interferon such as a tumor cytotoxic glycoprotein called CBF in Japanese Unexamined Patent Publication No. 146293 (1983), a tumor cell proliferation inhibitor (INF) having a molecular weight of 35,000-45,000 in Japanese Unexamined Patent Publication No. 33120 (1986), a tumor proliferation factor (FNF) in Japanese Unexamined Patent Publication No. 1872 (1986), a physiologically active substance having a molecular weight of 40,000-60,000 and an isoelectric point of pH 5.0±0.5 in Japanese Unexamined Patent Publication No. 103021 (1987), and a tumor cytotoxic factor having a molecular weight of 36,000±1,000 and a specific amino acid sequence at isoelectric point of pH 10.5

or higher in Japanese Unexamined Patent Publication No. 10998 (1989), have been known. The inventors have been investigating antitumor substances derived from human fibroblast cells and found a new antitumor proteinous substance. Furthermore, the inventors successfully cloned a cDNA coding for the protein and determined its amino acid sequence. Also the usefulness of the protein was confirmed. The new antitumor protein and its gene were disclosed in the inventors' International Patent Publication No. 10651 (1990). The new antitumor protein was named TCF-Ⅱ.

TCF-Ⅱ has both potent antitumor activity and proliferation stimulative activity for normal cells and is a member of the HGF (hepatocyte growth factor) group. Molecular weight determination of TCF-Ⅱ with SDS electrophoresis showed 78,000±2,000 or 74,000±2,000. The reduction products of TCF-Ⅱ showed a common band (A chain) at 52,000±2,000, and two bands (B and C chains) at 30,000±2,000 and 26,000±2,000, respectively.

TCF-Ⅱ may be applied for the regeneration of liver after the hepatectomy due to its proliferative effect for hepatocytes. This is now under investigation but no animal experiments confirming the effect or indicating use for the treatment of liver diseases have been known.

Moreover, improvement or therapeutic effect in hypoproteinemia due to liver diseases, renal insufficiency or undernutrition has ever been found. Plasma protein is

composed of more than 80 kinds of protein and most of their molecular weights range within 40,000-1,000,000. They often combine with carbohydrates or lipids to form conjugated proteins. These plasma proteins have important physiological significances such as blood coagulation factors, immunoglobulins, complements and enzymes, and also participate in the maintenance of osmotic pressure of plasma colloids and metabolism in the peripheral tissue. Most plasma proteins other than immunoglobulins are synthesized in the liver, released in the blood stream and widely distributed not only in the blood vessels but also in tissues and body cavity fluids, and actively exchange each other through lymph. Their catabolism includes secretion or excretion in gastrointestinal tract, kidneys, respiratory organs, reproductive organs and tear fluid; and degradation in the liver and reticuloendothelial system. The biological half life of plasma proteins is generally 2-20 days. The clinically serious hypoproteinemia occurs by various combinations of widely spread hemorrhage including ulcer and hematuria, decreased protein synthesis due to liver diseases, exhaustion and decreased production of plasma proteins accompanied by undernutrition due to all of nephrosis and nephritis, multiorgan failure, malignant tumors, infectious diseases, diabetes mellitus, gestosis and so forth.

Heretofore, intravenous administration of albumin

preparations has been performed for the treatment of above mentioned hypoproteinemia providing a temporary improvement but neither complete remission nor effective treatment has been found.

Furthermore, therapeutic effect of TCF-II for the treatment of wounds has not been found.

Skin damage due to wounds and burns has been palliatively treated by external application or oral administration of antibiotics, spreading of acrinol・zinc oxide oil or covering with chitin fibers or lyophilized porcine dermis or by surgical treatments such as sutures and skin grafts. Administration of epidermal cell growth factor (EGF) for the regeneration of skin tissue by the proliferation of epithelial cells has been tried but no definite treatment has been established.

Summary of the Invention

The inventors noticed the biological activity of TCF-II and have been investigating the use of TCF-II as an antitumor agent and diagnostic marker of the diseases.

The inventors found that TCF-II provides not only proliferation of hepatocytes but also therapeutic effects on various liver diseases. Heretofore, the therapeutic effect of TCF-II on various liver diseases had not been confirmed.

An object of the present invention is to provide a liver disease treatment agent comprising an effective ingredient

of TCF-Ⅱ.

The inventors found that TCF-Ⅱ exhibits therapeutic effect on various hypoproteinemia and the therapeutic effect of TCF-Ⅱ on hypoproteinemia is surprising.

Another object of the invention is to provides a highly effective protein synthesis stimulator comprising an effective ingredient of TCF-Ⅱ for the treatment of hypoproteinemia.

Furthermore, the inventors discovered that TCF-Ⅱ not only provides proliferation of hepatocytes, but also stimulates the proliferation of epithelial and fibroblast cells to accelerate the healing of wounds and burns.

Therefore, one object of the present invention is to provide a wound healing agent comprising an effective component of TCF-Ⅱ.

Thus, the present invention relates to a novel liver disease treatment agent comprising an effective component of TCF-Ⅱ; the agent provided by the present invention may be used for the treatment of liver diseases including acute and chronic hepatitis, cirrhosis of the liver or cholestatic liver diseases.

Furthermore, the present invention relates to a protein synthesis stimulator for the treatment of hypoproteinemia

containing TCF-Ⅱ as an effective component. These hypoproteinemia may be caused by liver diseases and leaky or undernutritional renal insufficiency. The present invention can be used for the stimulation of protein synthesis for the treatment of these hypoproteinemia.

Additionally, the present invention relates to a wound healing agent comprising an effective ingredient of TCF-Ⅱ. These lesions include not only injuries caused by incision but also burns, and the present invention can be applied for the treatment of these lesions.

Brief Description of the Drawings

Fig. 1 shows the results of thrombotest in liver resected pathologic rats.

Fig. 2 shows the serum fibrinogen level in liver resected pathologic rats.

Fig. 3 shows the concentration of serum triglyceride in liver resected pathologic rats.

Fig. 4 shows the content of total serum protein in liver resected pathologic rats.

Fig. 5 shows the weight of liver in liver resected pathologic rats.

Fig. 6 shows the content of liver protein in liver resected pathologic rats.

Fig. 7 shows the content of liver DNA in liver resected pathologic rats.

Fig. 8 shows the concentration of total serum protein in liver resected and intermittently or continuously TCF-Ⅱ administered rats.

Fig. 9 shows the concentration of serum albumin in liver resected and intermittently or continuously TCF-Ⅱ administered rats.

Fig. 10 shows the concentration of serum HDL-cholesterol in liver resected and intermittently or continuously TCF-Ⅱ administered rats.

Fig. 11 shows the concentration of serum triglycerides in liver resected and intermittently or continuously TCF-Ⅱ administered rats.

Fig. 12 shows the regeneration rate of liver resected and intermittently or continuously TCF-Ⅱ administered rats.

Fig. 13 shows the thrombotest value in liver resected and intermittently or continuously TCF-Ⅱ administered rats.

Fig. 14 shows the increase of total serum protein in normal rats administered with the treatment agent of the present invention.

Fig. 15 shows the increase of serum albumin in normal rats administered with the treatment agent of the present invention.

Fig. 16 shows the increase of total plasma fibrinogen in hypoproteinemia rats after 70% liver resection followed by administration of the treatment agent of the present invention.

Fig. 17 shows the increase of total serum protein in hypoproteinemia rats after 70% liver resection followed by administration of the treatment agent of the present invention.

Fig. 18 shows the shortening of prothrombin time in hypoproteinemia rats after 70% liver resection followed by administration of the treatment agent of the present invention.

Fig. 19 shows the increase of plasma fibrinogen in hypoproteinemia rats due to DIC followed by administration of the treatment agent of the present invention.

Fig. 20 shows the increase of antithrombin Ⅲ in hypoproteinemia rats caused by DIC followed by administration of the treatment agent of the present invention.

Fig. 21 shows the increase of total serum protein in hypoproteinemia rats caused by DIC followed by administration of the treatment agent of the present invention.

Fig. 22 shows the increase of total serum protein in hypoproteinemia rats caused by chronic renal failure followed by administration of the treatment agent of the present invention.

Fig. 23 shows the shortening of prothrombin time in hypoproteinemia rats caused by undernutrition followed by administration of the treatment agent of the present

invention.

Fig. 24 shows the increase of antithrombin Ⅲ activity in hypoproteinemia rats caused by undernutrition followed by administration of the treatment agent of the present invention.

In the Figs., * indicates $P<0.05$ and ** indicates $P<0.01$.

Detailed Explanation of Preferred Embodiments

The effective ingredient of the present invention is a known glycoprotein (TCF-Ⅱ) derived from human fibroblast cells as described previously.

TCF-Ⅱ showed a molecular weight of 78,000±2,000 or 74,000±2,000 in the non-reduced state, and a common band A of 52,000±2,000 and two bands B of 30,000±2,000 and C of 26,000±2,000 in the reduced state by SDS electrophoresis. TCF-Ⅱ also showed an isoelectric point at pH 7.4-8.6 and was determined as a glycoprotein having a 723 amino acid sequence.

The above mentioned TCF-Ⅱ can be obtained by evaporation of a human fibroblast cell culture solution, adsorption in an ion exchange resin and affinity chromatography of the eluate (WO90/10651) or by a genetic engineering method (WO92/01053).

TCF-Ⅱ can be obtained from human fibroblast cells cultured by the method disclosed in WO90/10651.

Furthermore, TCF-Ⅱ produced by a genetic recombination technique using microorganisms or other cells by the gene sequence disclosed in the above mentioned patent publication may be used. The production of TCF-Ⅱ by the genetic engineering method may be carried out by the method invented by the present inventors and disclosed in WO92/01053. In addition, TCF-Ⅱ analogues having different sugar chains or no sugar moieties produced by different host cells or microorganisms may also be used. However, presence of sugar moieties is preferable because of their participation in the in vivo metabolic rate.

TCF-Ⅱ can be concentrated and purified by conventional isolation and purification methods, for example, precipitation with an organic solvent, salting out, gel filtration chromatography, affinity chromatography using a monoclonal antibody and electrophoresis. The purification by affinity chromatography using a monoclonal antibody disclosed in Japanese Patent Application No. 177236 (1991) by the present inventors may be applied.

The resultant purified TCF-Ⅱ may be kept under lyophilization or deep freezing.

The liver disease treatment agent of the present invention may be intravenously, intraarterially, intramuscularly or subcutaneously administered as injection preparations. Drugs used for the treatment of liver diseases such as amino acids, vitamins, phospholipids, malotilate, prednisolone and

glycyrrhizin may be used concurrently.

Furthermore, the protein synthesis stimulator of the present invention may be administered as injection preparations and any route such as intravenous, intraarterial, intramuscular and subcutaneous injections can be selected. Blood coagulants such as fibrinogen, coagulation controlling factors such as antithrombin Ⅲ and drugs such as FOY, a protease inhibitor gabexate mesylate, used for the treatment of DIC are also used concurrently.

The injection preparations of TCF-Ⅱ may be used singly or in combination with above mentioned medicines and adjuvants such as human serum albumin, surface active agents, amino acids and sugars.

Additionally, the wound treatment agent of the present invention can be administered around the wound as injection preparations. Direct spreading of TCF-Ⅱ or homogenous ointments containing TCF-Ⅱ prepared with fat, fatty oil, lanolin, paraffin, wax, resin, glycols, higher alcohols, glycerin, water, an emulsifier, a suspending agent and so forth may be used. Furthermore, plasters, aerosols, liniments and so forth may be prepared. TCF-Ⅱ may be adsorbed in sterilized gauze, lyophilized porcine epidermis or chitin fiber used for the protection of wounded skin surface. TCF-Ⅱ may be administered together with antibiotics, antibacterials and antiseptics, if necessary. Administration to the surgical site of suture or skin graft

may be considered.

The wound treatment agent of the present invention can be applied for the treatment of a variety of skin lesions including bedsores, incision in operation, burns, traumatic skin defects in addition to common incised or lacerated wounds.

The doses of TCF-Ⅱ included in the liver disease treatment agents, protein synthesis stimulants and abnormal blood coagulation treatment agents may be determined according to the symptoms, conditions and age of the patients but preparations containing 100-30,000 μg, preferably 500-3,000 μg of TCF-Ⅱ are generally administered 1-7 times a week. Chronic administration may be appropriate according to the symptoms and conditions of the patients.

The doses of TCF-Ⅱ included in the wound treatment agents of the present invention can be determined according to the symptoms and conditions of the patients and generally administered at doses of 100-30,000 μg, preferably, 500-3,000 μg of TCF-Ⅱ to the wound 1-7 times a week. Long term administration may be used according to the symptoms and conditions of the patients.

The present invention will be explained in more detail by the following examples.

Example 1.

Purification of TCF-Ⅱ

Purified TCF-Ⅱ was obtained by cell culture according to the method disclosed in WO90/10651 or Higashio, K. et al. (B.B.R.C., 170, 397-404, 1990).

Human fibroblast cells IMR-90 (ATCC CCL 186), 3 x 10^6 cells, were inoculated in 100 ml of DMEM medium containing 5% bovine serum in a roller bottle and cultured at rotations of 0.5-2/min. for seven days. The culture was continued up to 1 x 10^7 cells in total, the proliferated cells were separated by treatment with trypsin and collected at the bottom of the bottle. In the bottle, 100 g of sterilized 5-9 mesh ceramic (Toshiba Ceramic Co., Ltd.) was placed and cultured for 24 hrs. upon standing. Then, 500 ml of the culture medium shown above was added to the bottle and cultured further. The total culture medium was recovered every 7-10 days and fresh culture medium was supplied for further culture. Thus, the culture was continued for two months and four l/bottle of the culture solution was recovered.

The combined culture solution showed specific activity of 32 μ/ml.

Ultrafiltration of 750 l of the cultured solution was performed using a membrane filter (Amicon Corp., MW 6,000 cut) and the filtrate was chromatographed in five steps using CM Sephadex* C-50 (Farmacia Biosystems Corp.), ConA

*-trademark

Sepharose (Farmacia Biosystems Corp.), MonoS column (Farmacia Biosystems Corp.) and heparin Sepharose* (Farmacia Biosystems Corp.) to give purified TCF-Ⅱ having specific activity of 5,248,000 U/mg.

Example 2

Production of gene recombinant TCF-Ⅱ

TCF-Ⅱ gene recombinant cells were cultured according to the method disclosed in WO92/01053 and purified TCF-Ⅱ was obtained. Transformed Namalwa cells were cultured and 20 l of the culture solution was obtained. The culture solution was treated successively with HPLC using CM-Sephadex C-50 chromato column, Con-A Sepharose CL-6B chromato column and MonoS* column to give approximately 11 mg of active TCF-Ⅱ.

Example 3

Production of pharmaceutical compositions of TCF-Ⅱ

In the present examples, recombinant TCF-Ⅱ obtained by Example 2 was used for the production of intravenous, subcutaneous and intramuscular injection preparations.

(1) TCF-Ⅱ 40 μg

Human serum albumin 1 mg

The above composition was dissolved in 0.01M PBS at pH 7.0 and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

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(2) TCF-II 40 μg

Tween 80* 1 mg

Human serum albumin 100 mg

This composition was dissolved in a saline solution for injection and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed..

(3) TCF-II 20 μg

Tween 80 2 mg

Sorbitol 4 g

This composition was dissolved in 0.01M PBS at pH 7.0 and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

(4) TCF-II 40 μg

Tween 80 2 mg

Glycine 2 g

This composition was dissolved in a saline solution for injection and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

(5) TCF-II 40 μg

Tween 80 1 mg

Sorbitol 2 g

Glycine 1 g

This composition was dissolved in a saline solution for injection and adjusted to 20 ml in total. The solution was

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sterilized, divided into vials (2 ml each), lyophilized and sealed.

(6) TCF-II	20 μg
Sorbitol	4 g
Human serum albumin	50 mg

This composition was dissolved in 0.01M PBS at pH 7.0 and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

(7) TCF-II	40 μg
Glycine	2 g
Human serum albumin	50 mg

This composition was dissolved in a saline solution for injection and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

(8) TCF-II	10 mg
Human serum albumin	100 mg

This composition was dissolved in 0.01M PBS at pH 7.0 and adjusted to 20 ml in total. The solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

Preparation of TCF-II ointment

(9) TCF-II	1,000 mg
Purified lanolin	20 g
White soft paraffin	80 g

TCF-II was mixed with a small amount of purified water, and mixed and kneaded portionwise with lanolin. White soft

paraffin was added portionwise to the resultant mixture and kneaded to give a TCF-Ⅱ ointment.

(10) TCF-Ⅱ 1,000 mg

Macrogol 400 5 ml

Macrogol ointment 100 g

TCF-Ⅱ was mixed with a small amount of purified water, and mixed and kneaded portionwise with Macrogol 400. Macrogol* ointment was added portionwise to the resultant mixture and kneaded to give a TCF-Ⅱ ointment.

These pharmaceutical preparations can be used as liver disease treatment agents, protein synthesis stimulants, and wound healing agents according to the above mentioned dosage and regimen.

Liver disease treatment agents containing TCF-Ⅱ as an effective ingredient are provided by the present invention. Hereinafter, test experiments with treatment agents prepared according to the present invention will be shown to confirm the therapeutic effects and explain the present invention.

Experiment 1

Therapeutic effect on acute liver disease, chronic liver disease and liver cirrhosis

(1) Method

Therapeutic effects on 70% resected normal liver, acute liver diseases (hepatitis type and hepatocyte type), chronic

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liver diseases and liver cirrhosis were confirmed.

Male Wistar rats, seven week old and average body weight of 200 g were used for the experiments. Normal liver rats were prepared by resection of 70% of the liver (n = 6). Acute hepatitis rats were prepared by subcutaneous administration of 500 μg/kg of galactosamine immediately after the resection (n = 9-10). Acute hepatocyte damaged rats were prepared by oral administration of 0.3 ml/kg of carbon tetrachloride immediately after the resection (n = 10). Rats were repeatedly orally administered 0.7 ml/kg of carbon tetrachloride twice a week for 4 and 10 weeks, and their livers were similarly resected to prepare chronic liver damaged rats (n = 10) and liver cirrhosis rats (n = 9-10), respectively. In chronic liver damaged rats, 0.3 ml/kg of carbon tetrachloride was orally administered immediately after the resection of the liver to cause similar acute symptoms to those of acute hepatocyte damaged rats and the response to TCF-II was comparatively investigated.

TCF-II was intravenously administered every 12 hrs. immediately after the resection of liver, at doses of 20, 100, and 500 μg/kg, respectively, in normal rats and 500 μg/kg in the other rat groups, and the responses were compared to those of 0.1% human serum albumin added PBS administration group.

Responses to the action of TCF-II in normal liver resected rats were investigated by determination of thrombotest value

and serum glyceride concentration 48 hrs. after the resection and total serum protein and serum HDL cholesterol concentrations 72 hrs. after the resection and liver weight, liver DNA and liver total protein concentrations 96 hrs. after the resection, respectively.

(2) Results

Delayed thrombotest value, decrease of serum triglyceride, total serum protein and HDL cholesterol were observed in 70% liver resected rats, but TCF-II dose dependently improved all these parameters.

In normal liver, acutely damaged liver, chronically damaged liver and liver cirrhosis rats, TCF-II administration groups improved the parameters of thrombotest value (Fig. 1), plasma fibrinogen concentration (Fig. 2), serum triglyceride concentration (Fig. 3) and total serum protein concentration (Fig. 4), liver weight (Fig. 5), liver total protein concentration (Fig. 6) and liver DNA concentration (Fig. 7) more than those of 0.1% human serum albumin added PBS administration group.

Experiment 2

Effect on fatty liver

(1) Method

To seven week old male Wistar rats, 250 mg/kg of DL-ethionine was intraperitoneally administered successively for four days (n = 10). TCF-II was intravenously

administered to these rats at doses of 50 and 500 μg/kg every 12 hrs. and prothrombin time (pT), antithrombin Ⅲ activity (AT Ⅲ), blood urea nitrogen (BUN), total cholesterol (T-CHO), phospholipid (PL), HDL cholesterol (HDL) and mitotoxic index to 1,000 hepatocytes after 48 hrs., and total serum protein (TP), transaminase (GOT) and lactic acid dehydrogenase (LDH) after 72 hrs. were determined.

(2) Results

The average values and their standard errors are shown in Table 1. TCF-Ⅱ dose dependently improved symptoms of ethionine induced fatty liver rats at doses of 50 μg/kg or over.

Table 1 TCF-Ⅱ administered hepatic parameters in ethionine induced fatty liver rats

	Normal group	Solvent	50 μg/kg	500 μg/kg
After 48 hrs.				
PT (sec)	14.43 ±0.13**	17.28 ±0.48	16.57 ±0.27	15.78 ±0.28**
FIB (g/L)	1.83 ±0.03	1.79 ±0.11	2.12 ±0.05**	2.25 ±0.05**
TT (sec.)	23.58 ±0.28**	32.34 ±1.66	30.00 ±0.76	28.16 ±0.75*
ATⅢ (%)	128.7 ±2.5**	106.7 ±4.0	120 ±4.1*	131.1 ±3.8**
UN (mg/dl)	18.7 ±0.4**	30.6 ±3.2	30.8 ±3.0	23.9 ±0.9**
T-CHO (mg/dl)	78.1 ±2.9**	57.3 ±3.9	73.2 ±4.1*	96.2 ±6.7**
PL (mg/dl)	152.1 ±4.3*	134.0 ±8.3	157.4 ±4.4**	188.0 ±0.6 **
HDL (mg/dl)	39.6 ±1.5	15.3 ±2.1	18.6 ±2.5	23.1 ±3.1
M.I. (/1,000 cells)	5.2 ±1.1**	0.1 ±0.1	0.4 ±0.2	2.9 ±0.9*
Liver weight (g/100 g B.W.)	4.85 ±0.09	4.66 ±0.18	4.89 ±0.08	4.84 ±0.08
After 72 hrs.				
TP (mg/dl)	5.81 ±0.08**	5.77 ±0.09	6.08 ±0.08	6.23 ±0.08**
GOT (IU/L)	68.3 ±2.8*	93.9 ±5.7	77.2 ±3.3	60.1 ±2.3**
LDH (IU/L)	1499 ±190*	2605 ±256	1985 ±126	1400 ±121**

**: Significant at P=0.01 to solvent group
*: Significant at P=0.05 to solvent group

Experiment 3

Effect on cholestatic liver damage

(1) Method

To seven week old male Wistar rats, 50 mg/kg of α-

naphthyl isothiocyanate was orally administered. TCF-Ⅱ was intravenously administered twice to these rats immediately after the administration of α-naphthyl isothiocyanate and after 12 hrs. Blood was drawn 12 hrs. after the administration of TCF-Ⅱ and serum transaminase (GOT, GPT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), total bilirubin (T-BIL), direct bilirubin (D-BIL) and the excretion of foreign materials by liver (BSP test) were measured.

(2) Results

The average values and standard errors are shown in Table 2. TCF-Ⅱ dose dependently improved all parameters at doses of 500 μg/kg.

Table 2 TCF-Ⅱ administered hepatic parameters in cholestatic damaged liver rats

	Normal group	Solvent	50 μg/kg	500 μg/kg
GOT(IU/L)	71.2 ±3.1**	230.1 ±25.6	211.9 ±18.9	183.7 ±26.3
GPT(IU/L)	24.6 ±1.1**	62.9 ±8.0	56.6 ±5.4	48.0 ±7.8
ALP(IU/L)	609.7 ±35.0**	1034.7 ±63.6	1088.8 ±77.3	839.8 ±70.0
γ-GTP (IU/L)	2.2 ±0.2**	11.5 ±0.8	12.2 ±0.9	8.3 ±0.7**
T-Bil (mg/dl)	0.22 ±0.11**	2.05 ±0.1	2.09 ±0.1	1.60 ±0.12*
D-Bil (mg/dl)	0.15 ±0.01**	1.18 ±0.0	1.19 ±0.0	0.93 ±0.08*
BSP retention rate (%)	0.50 ±0.02**	29.38 ±0.51	——— ———	24.76 ±1.87**

**: Significant at P=0.01 to solvent group
*: Significant at P=0.05 to solvent group

Experiment 4

Effect on acute hepatitis

(1) Method

To seven week old male Wistar rats (n = 8), one g/kg of galactosamine was orally and subcutaneously administered. TCF-II was intravenously administered four times to these rats starting immediately after the administration of galactosamine every four hrs. for two days. Blood was collected 48 hrs. after the administration of galactosamine and serum transaminase (GOT, GPT), γ-glutamyl transpeptidase (γ-GTP), total serum protein (TP), total cholesterol (T-CHO), triglyceride (TG), phospholipid (PL), β-lipoprotein (β-Lipo) and liver weight, liver protein concentration and liver DNA concentration were determined.

(2) Results

The average values and their standard errors are shown in Table 3. TCF-II improved all parameters at a dose of 250 μg/kg and improved the symptoms of acute hepatitis.

Table 3. TCF-Ⅱ administered hepatic parameters in galactosamine induced acute hepatitis rats

	Normal group	Solvent	250 μg/kg
GOT (IU/L)	69.4 ±3.1**	721.4 ±134.6	580.9 ±195.3
GPT (IU/L)	22.3 ±2.2**	219.5 ±43.5	189.4 ±62.9
γ-GTP (IU/L)	2.28 ±0.30**	8.31 ±1.50	6.94 ±1.84
TP (mg/dl)	5.46 ±0.08**	4.48 ±0.10	4.48 ±0.12
T-CHO (mg/dl)	71.5 ±2.5**	51.1 ±1.9	62.8 ±3.1*
TG (mg/dl)	99.4 ±15.2**	23.5 ±6.6	57.4 ±20.9
PL (mg/dl)	136.3 ±4.7**	108.9 ±3.9	135.7 ±7.0*
β-lipo (mg/dl)	145.6 ±18.1	98.5 ±7.6	149.0 ±23.1
Liver weight (g/100 g B.W.)	4.40 ±0.09**	3.86 ±0.10	4.14 ±0.12
Liver protein content (g/100 g B.W.)	0.56 ±0.02**	0.46 ±0.01	0.49 ±0.02
Liver DNA content (mg/100 g B.W.)	11.6 ±0.4**	13.2 ±0.5	13.9 ±0.4

**: Significant at P=0.01 to solvent group
*: Significant at P=0.05 to solvent group

Experiment 5

Therapeutic effect according to type of administration

(1) Method

Seven week old male Wistar rats, body weight 200 g, were used for the experiment and normal liver rat group was prepared by resection of 70% of the liver. The administration of TCF-Ⅱ was started immediately after the resection intravenously and intermittently at 12 hr.

intervals (n = 6) or by continuous infusion (n = 12) and the responses were compared. Both groups were administered at the same dose of one mg/kg/day. Total serum protein, albumin, triglyceride, HDL-cholesterol and thrombotest value 48 hrs. after the resection were determined and liver weight was determined to estimate the regeneration rate of the liver.

(2) Results

Changes of parameters by the administration of TCF-Ⅱ in 70% liver resected rats are shown in total serum protein (Fig. 8), albumin (Fig. 9), HDL-cholesterol (Fig. 10), triglyceride (Fig. 11), regeneration rate (Fig. 12) and thrombotest value (Fig. 13). All parameters showed improvement in the continuous drip infusion group.

Above results of experiments exhibit excellent effect of the agent of the present invention against heretofore intractable liver diseases.

The present invention provides protein synthesis stimulating agents comprising an effective ingredient of TCF-Ⅱ for the treatment of hypoproteinemia. Hereinafter, experiments exhibiting the therapeutic effect of the agent prepared by the present examples will be shown to explain the effect of the present invention.

Experiment 6

Total serum protein increasing effect

(1) Method

TCF-Ⅱ was repeatedly and intravenously administered to seven week old male Wistar rats, six in one group, at doses of zero, five, 50, 500 and 5,000 μg/kg at 12 hr. interval for 14 days (28 times in total) and blood was drawn 12 hrs. after the final administration to determine total serum protein and serum albumin.

(2) Results

TCF-Ⅱ dose dependently increased the total serum protein (Fig. 14) and serum albumin (Fig. 15). Particularly, doses of five μg/kg or over significantly increased both parameters confirming the increasing effect on serum protein.

Experiment 7.

Therapeutic effect of TCF-Ⅱ on hypoproteinemia due to liver resection

Plasma protein lowered model rats were prepared by liver resection and the effect of TCF-Ⅱ was confirmed using the model animals. In addition, plasma fibrinogen concentration and exogenous coagulation factor were noted as indicators of plasma protein and the changes in prothrombin time were noted as indicators of plasma protein including exogenous coagulation factors (Factors Ⅱ, Ⅴ, Ⅶ and Ⅹ).

(1) Method

Seven week old male Wistar rats, six in one group, were used for the experiment and 70% of the liver was resected. TCF-II was repeatedly and intravenously administered to the rats at doses of zero, 20, 100 and 500 μg/kg at 12 hr. intervals for two days, four times in total, and blood was drawn 48 hrs. after the start of the experiment to determine prothrombin time, plasma fibrinogen concentration and total serum protein.

(2) Results

Shortening of prothrombin time (Fig. 18), increases of plasma fibrinogen concentration (Fig. 16) and total serum protein (Fig. 17) were observed with the administration of TCF-II. These results indicate the efficacy of the present invention for the treatment of hypoproteinemia due to liver damage.

Experiment 8

Therapeutic effect on hypoproteinemia caused by DIC

DIC causes sudden increase in consumption of plasma protein due to intravascular coagulation and reveals hypoproteinemia. The therapeutic agent of the present invention was given to model animals to confirm the therapeutic effect.

(1) Method

Seven week old male Wistar rats, 10 in one group, were

used for the experiment and 70% of the liver was resected. Immediately after the resection, 500 mg/kg of galactosamine was subcutaneously administered to prepare pathologic model of both liver damage and DIC symptoms. TCF-II was repeatedly and intravenously administered to the rats at a dose of 500 μg/kg at 12 hr. intervals for two days, four times in total, and blood was drawn 48 hrs. after the start of the experiment to determine plasma fibrinogen concentration, antithrombin III activity and total serum protein as indicators for the hypoproteinemia.

(2) Results

Increases of plasma fibrinogen (Fig. 19), antithrombin III activity (Fig. 20) and total serum protein (Fig. 21) were observed with the administration of TCF-II, confirming the efficacy of the present invention for the treatment of hypoproteinemia caused by DIC.

Experiment 9

Therapeutic effect on leaky hypoproteinemia caused by renal failure

(1) Method

Eight week old male Wistar rats, 11 in one group, were used for the experiment and the kidneys were partially resected. After three weeks, TCF-II was repeatedly and intravenously administered to the rats at doses of zero and 500 μg/kg at 12 hr. intervals for 10 times in total, and

blood was drawn 12 hrs. after the administration to determine total serum protein concentration.

(2) Results

Increase of total serum protein was observed with the administration of TCF-Ⅱ, confirming the efficacy of the present invention for the treatment of leaky hypoproteinemia caused by renal failure (Fig. 22).

Experiment 10

Therapeutic effect on undernutritional hypoproteinemia

(1) Method

Essential amino acid deficient rats were prepared by replacing an essential amino acid methionine with a synthetic DL-ethionine.

Ethionine was repeatedly and intraperitoneally administered at a dose of 250 mg/kg/day for four days to prepare methionine deficient rat. The serum protein in the rats decreased due to amino acid deficiency. TCF-Ⅱ was repeatedly and intravenously administered to the rats, 10 rats in one group, at doses of zero, 50, and 500 μg/kg every 12 hrs. for two days and four times in total; blood was drawn 48 hrs. after the start of the experiment and the prothrombin time and antithrombin Ⅲ activity were determined as indicators of hypoproteinemia.

(2) Results

Shortening of prothrombin time (Fig. 23) and increase of

antithrombin Ⅲ activity (Fig. 24) were observed with the administration of TCF-Ⅱ, confirming the efficacy of the present invention for the treatment of hypoproteinemia due to undernutrition.

These results confirm the efficacy of the treatment agent of the present invention on hypoproteinemia.

The present invention provides a wound healing treatment agent containing an effective ingredient of TCF-Ⅱ. The present invention will be explained by the following experiments confirming the therapeutic effect of TCF-Ⅱ.

Experiment 11

① Test method

Eight week old male Wistar rats (Charles River Japan, Inc.), 8-9 animals in one group, were sheared on the back and incised approximately 1.5 cm length symmetrically to the median line. Then the incised site was treated with penicillin for the prevention of suppuration and sutured at one point in the center of the incision.

TCF-Ⅱ was dissolved in pyrogen free PBS containing 0.1% human serum albumin at a rate of one mg/ml and was directly spread on the site of incision immediately after the incision twice a day. The suture was removed on day four, rats were killed on day six and the skin was stripped off. A skin test sample was prepared by cutting the site of incision rectangularly. The obtained test sample was pulled

at both ends to determine its tensile force until the sutured site was broken. The determined value with FD pickup (Nihon Kohden Corp., TB-611T) was recorded in a polygraph (Nihon Kohden Corp., RM-6200) via an amplifier for strain pressure (Nihon Kohden Corp., AP-601G). The control group was prepared by spreading solely the solvent, 0.1% human serum albumin containing PBS, instead of the test solution.

② Test results

The maximum tension for the break of the suture is shown in Table 4.

Table 4 Tensile force of rat skin at the site of suture

Treatment	Tensile force (g) (Mean ± S.E.)
Control (n = 9)	190.06±26.2
TCF-Ⅱ (n = 8)	314.4 ±24.4**

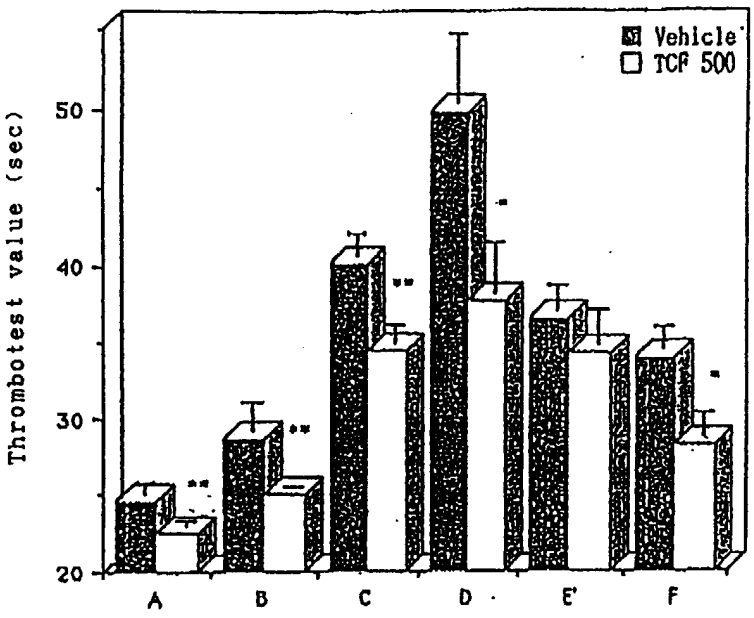
** P<0.01 (Wilcoxon test)

As shown in the Table 4, the bond strength at the site of incision in TCF-Ⅱ administered group was approximately 1.65 times that of the control group indicating a wound healing effect of TCF-Ⅱ.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

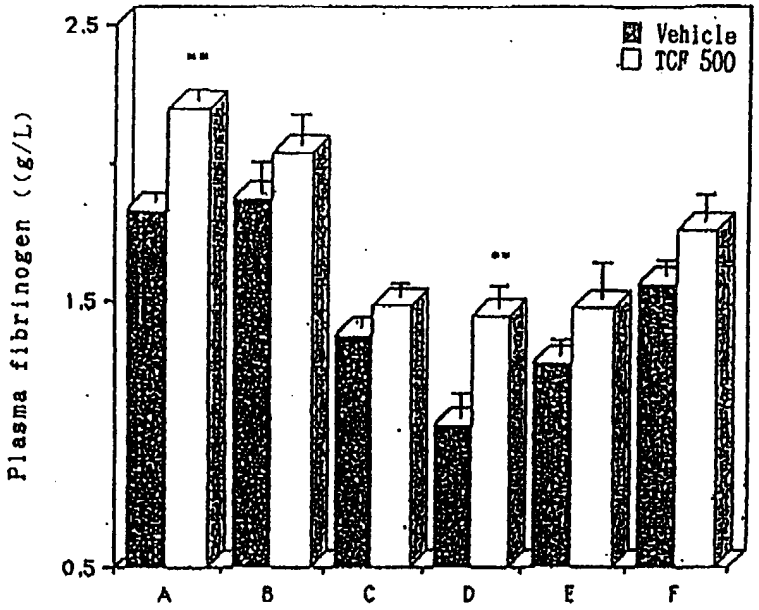
1. The use of TCF-II for the preparation of a medicament for the treatment of hypoproteinemia.

2. The use of TCF-II for the preparation of a medicament for the treatment of hypoproteinemia selected from hepatopathic hypoproteinemia, leaky hypoproteinemia caused by renal failure and undernutritional hypoproteinemia.



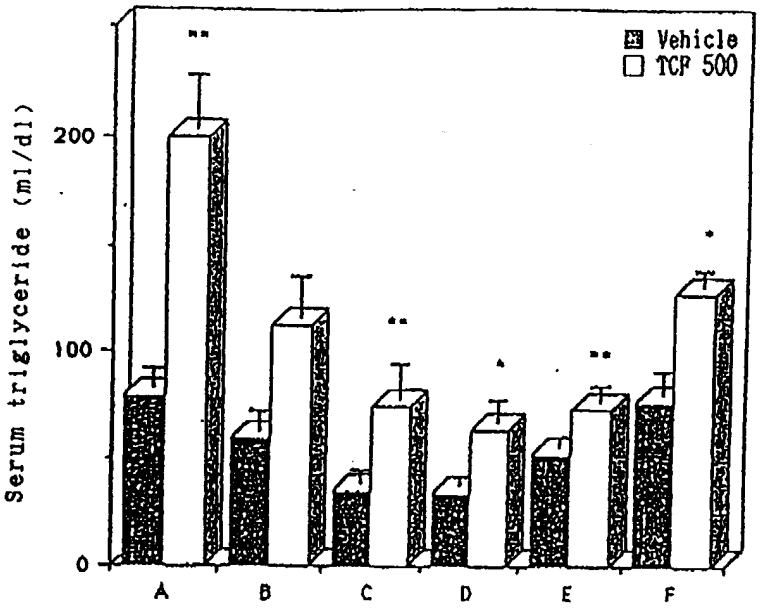
A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 1



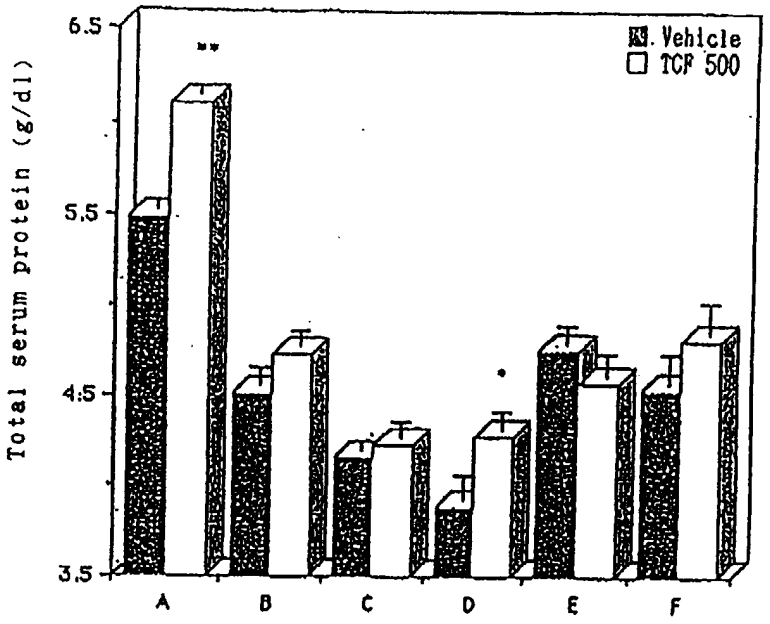
A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 2



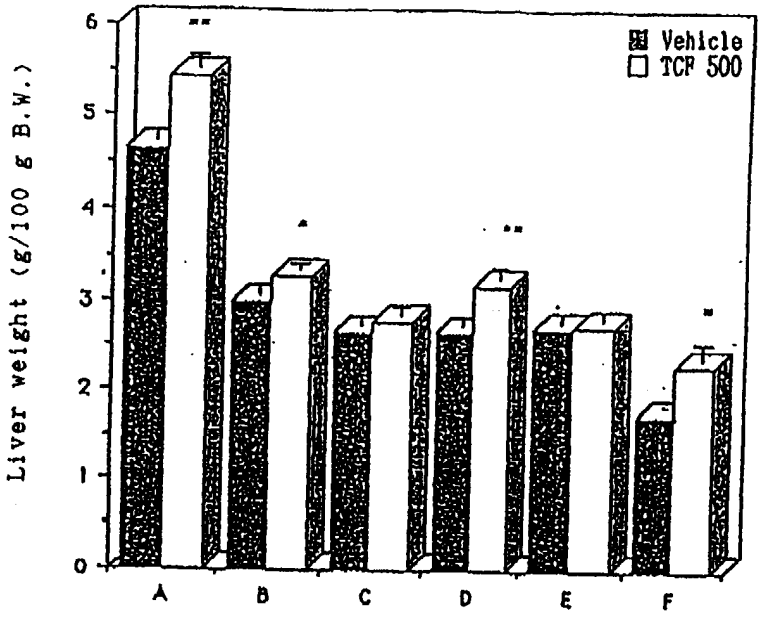
A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 3



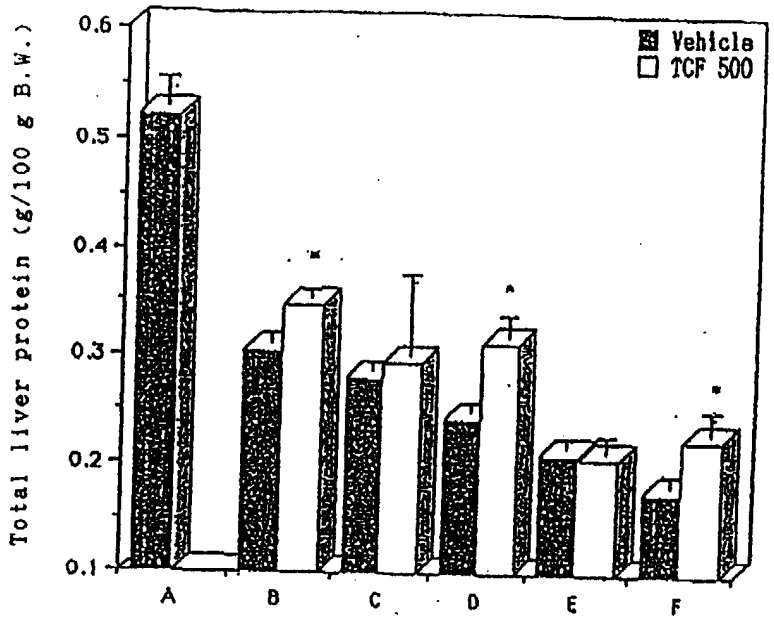
A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 4



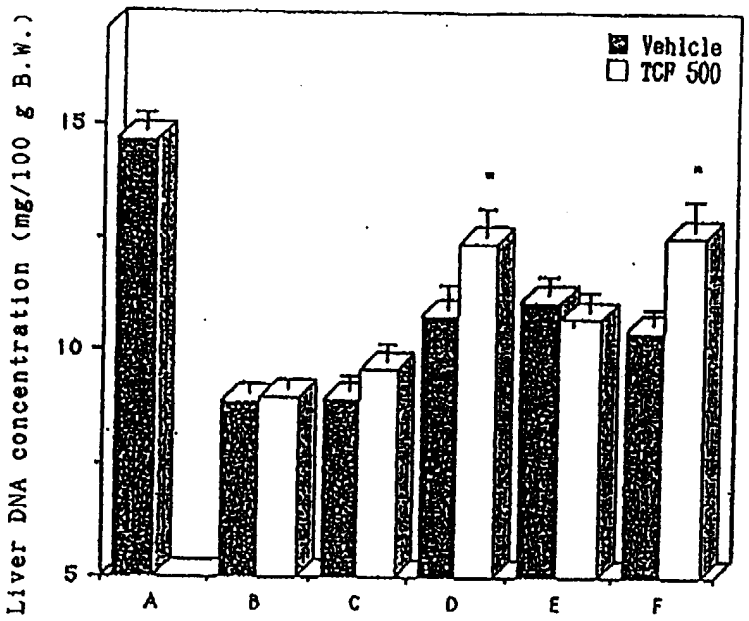
A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 5



A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 6



A; Normal liver
B: Normal liver resected
C: Normal liver resected + GAL
D: Normal liver resected + CCL_4
E: Chronically damaged liver resected + CCl_4
F: Cirrhosis of liver resected + CCl_4

Fig. 7

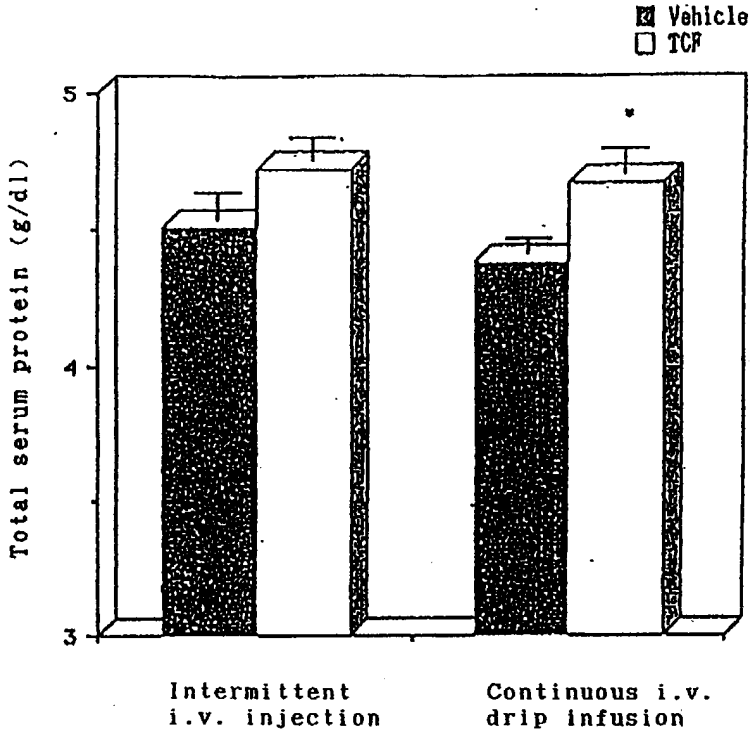

Vehicle
TCF
Total serum protein (g/dl)
5
4
3
*
Intermittent
i.v. injection
Continuous i.v.
drip infusion

Fig. 8

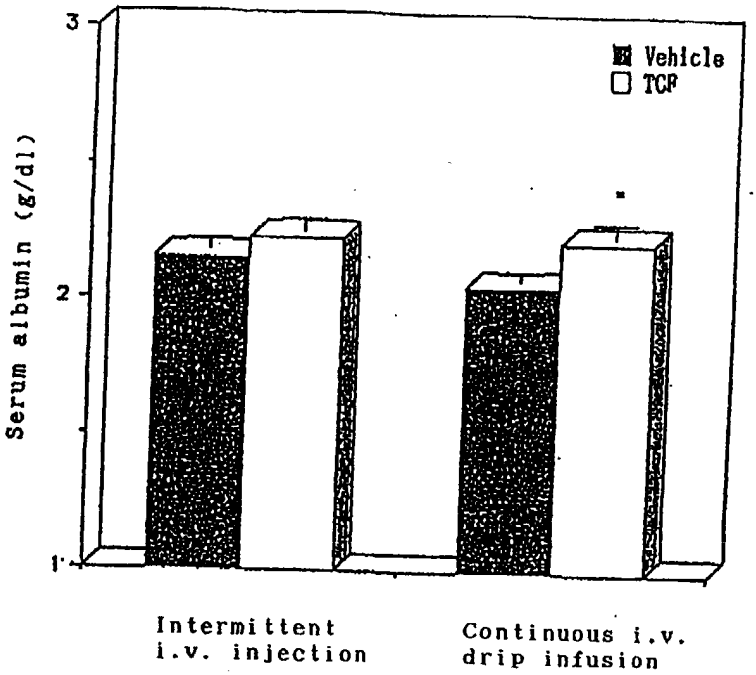

3
2
1
Serum albumin (g/dl)
Vehicle
TCP
Intermittent
i.v. injection
Continuous i.v.
drip infusion

Fig. 9

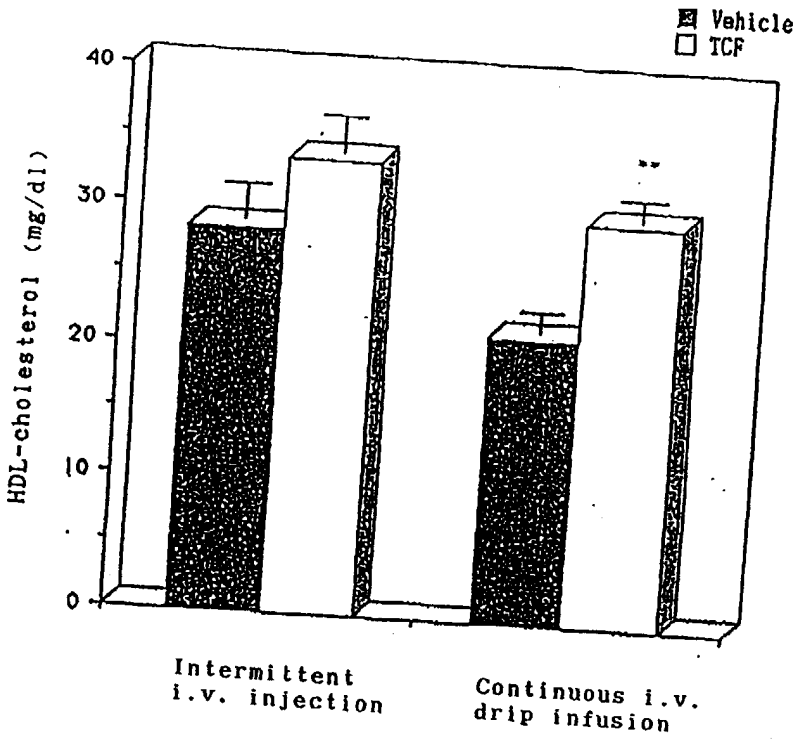

Vehicle
TCF
HDL-cholesterol (mg/dl)
40
30
20
10
0
**
Intermittent i.v. injection
Continuous i.v. drip infusion

Fig. 10

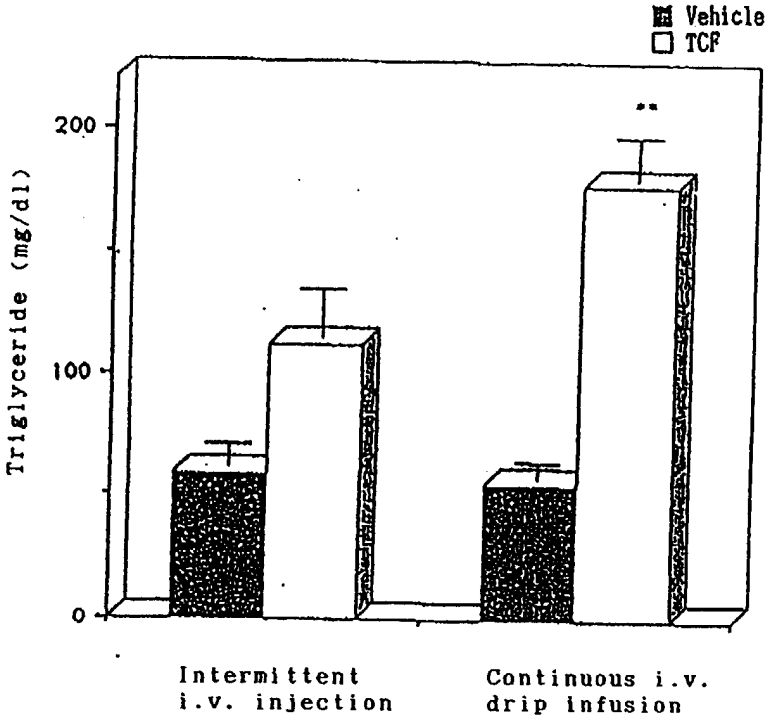

Vehicle
TCF
Triglyceride (mg/dl)
200
100
0
**
Intermittent
i.v. injection
Continuous i.v.
drip infusion

Fig. 11

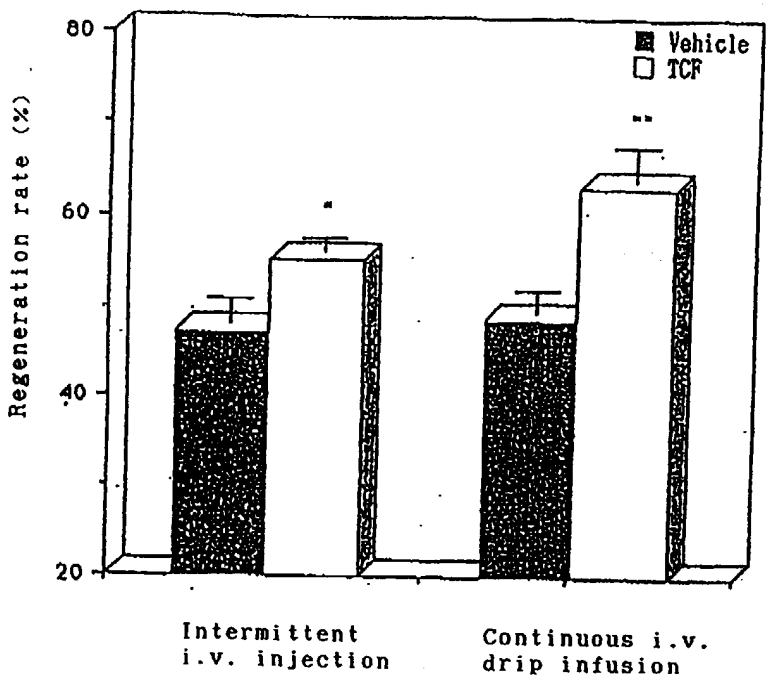

80
60
40
20
Regeneration rate (%)
Vehicle
TCF
*
**
Intermittent
i.v. injection
Continuous i.v.
drip infusion

Fig. 12

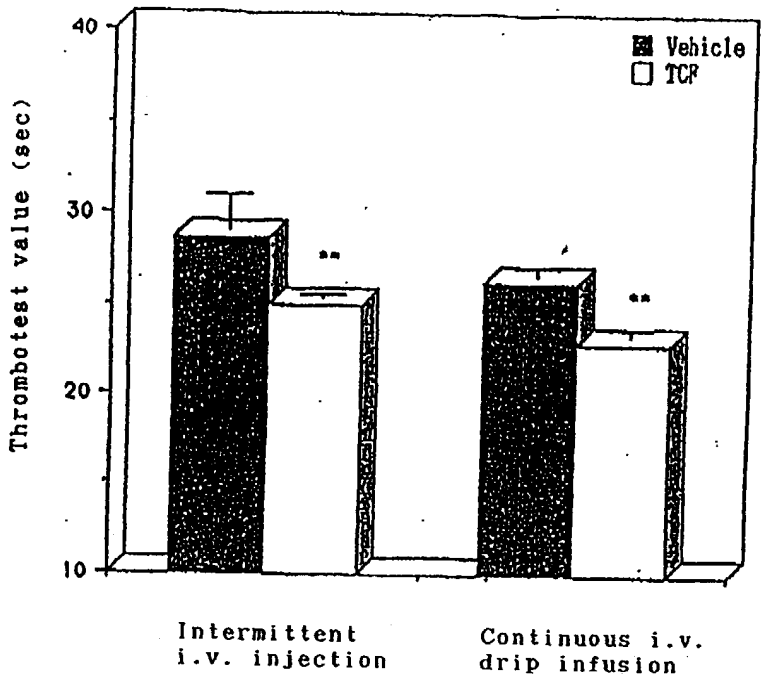

40
30
20
10
Thrombotest value (sec)
Vehicle
TCR
**
**
Intermittent
i.v. injection
Continuous i.v.
drip infusion

Fig. 13

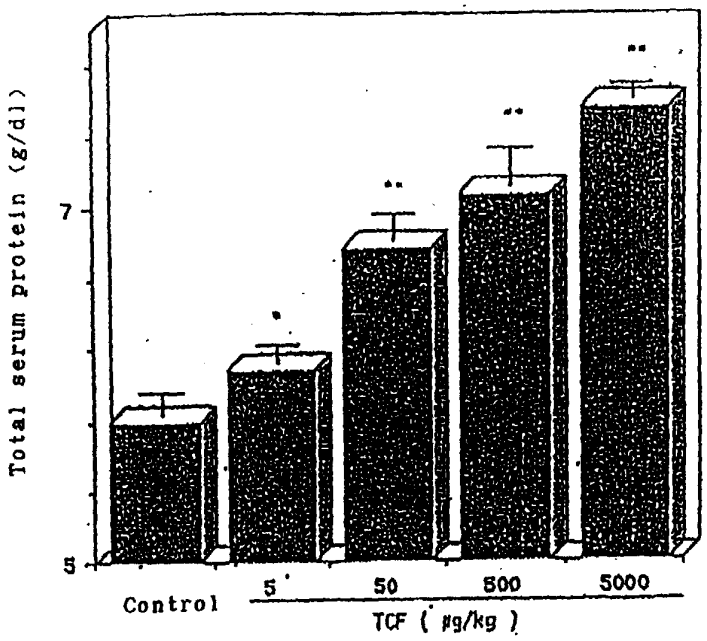


Fig. 14

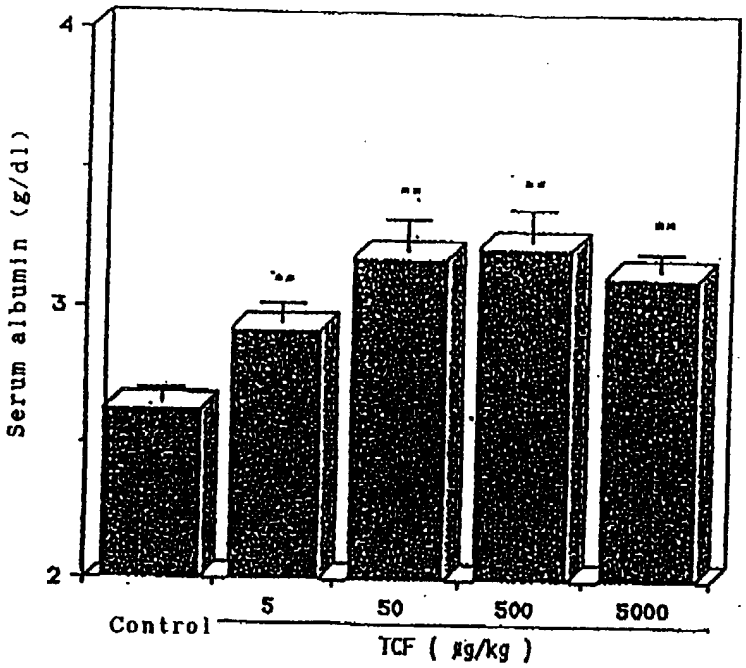


Fig. 15

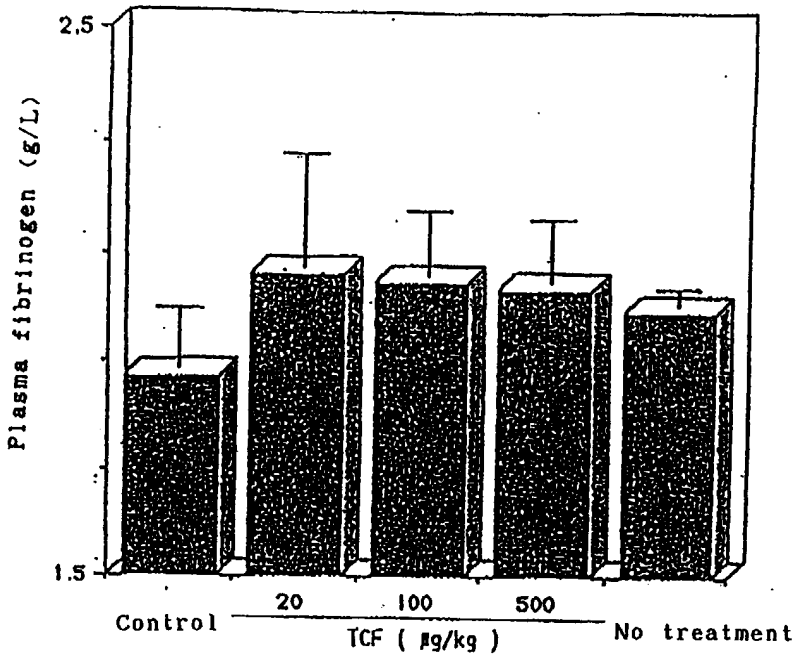

2.5
1.5
Plasma fibrinogen (g/L)
Control
20
100
500
TCF (μg/kg)
No treatment

Fig. 16

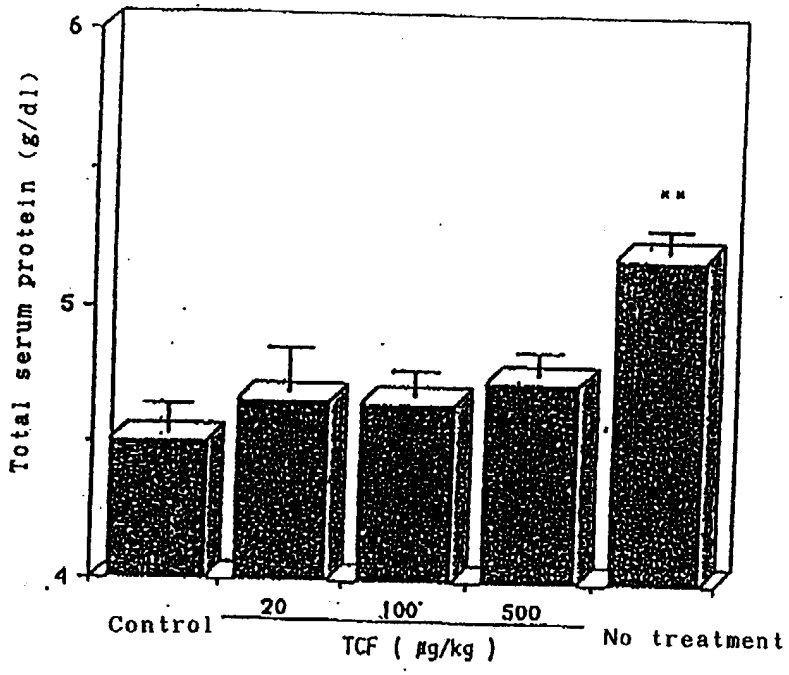

Total serum protein (g/dl)
6
5
4
**
Control
20
100
500
No treatment
TCF (μg/kg)

Fig. 17

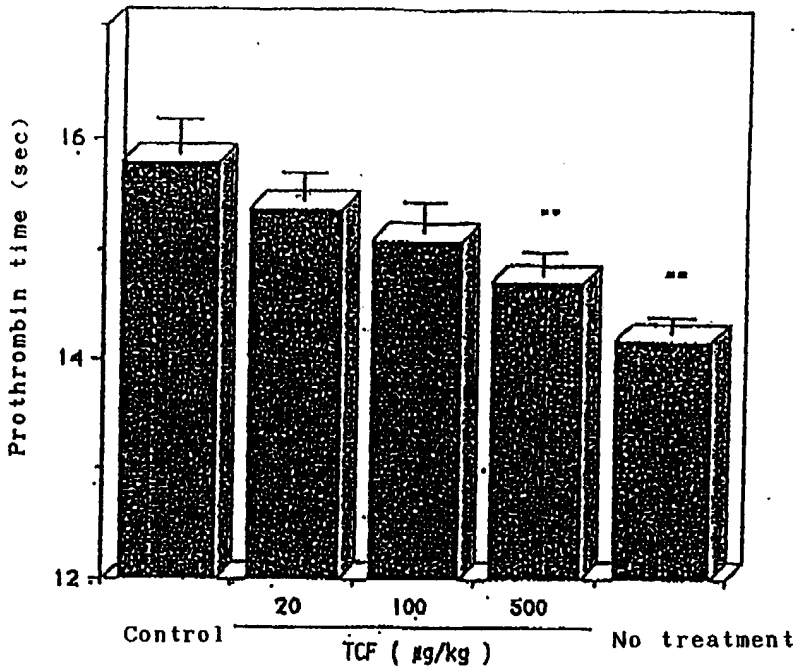

Prothrombin time (sec)
16
14
12
**
**
20
100
500
Control
No treatment
TCF (μg/kg)

Fig. 18

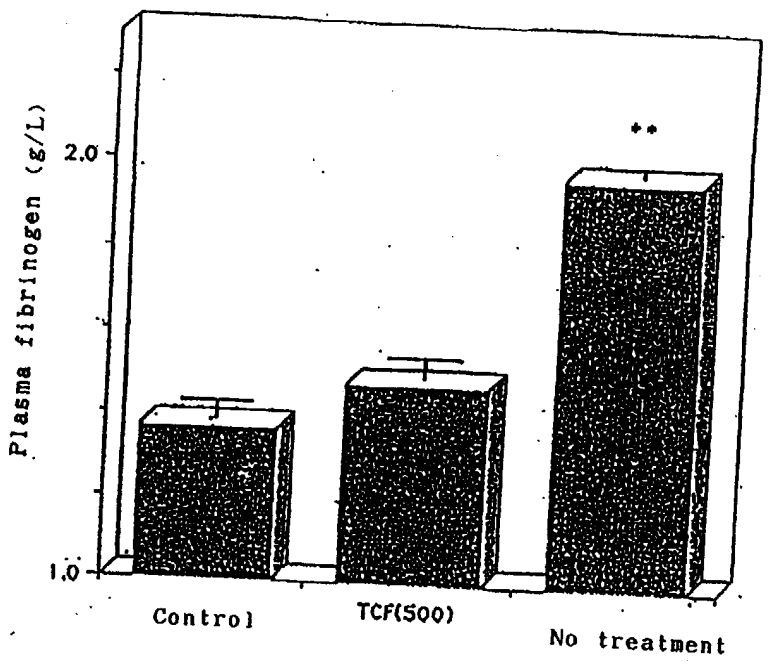


Fig. 19

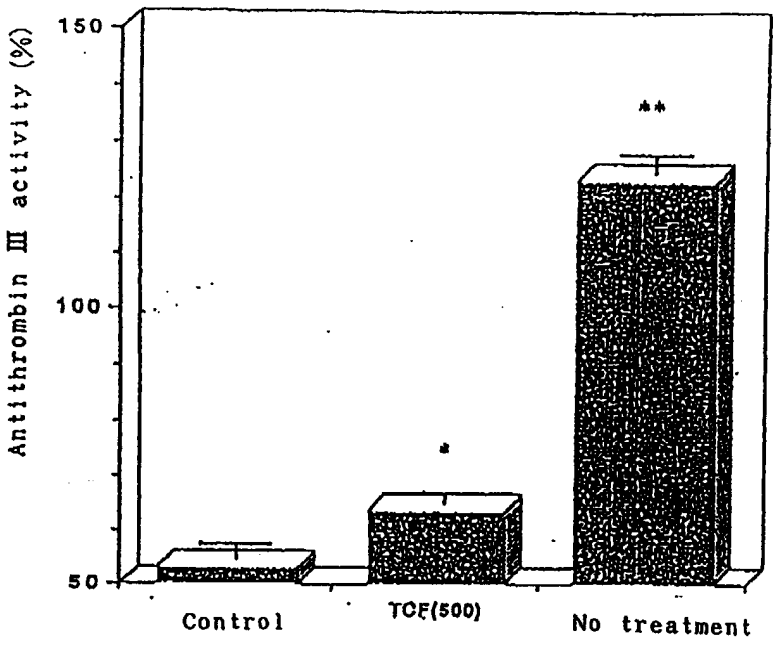

Antithrombin III activity (%)
150
100
50
*
**
Control
TCF(500)
No treatment

Fig. 20

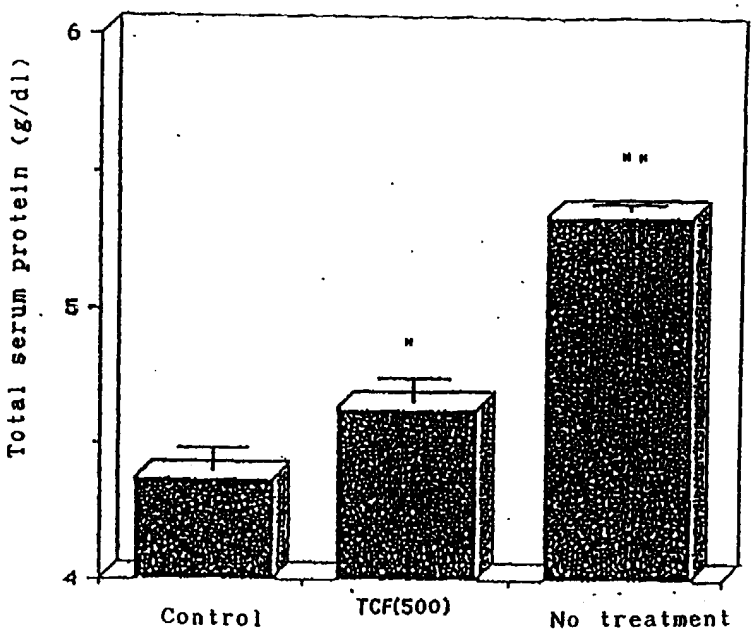


Fig. 21

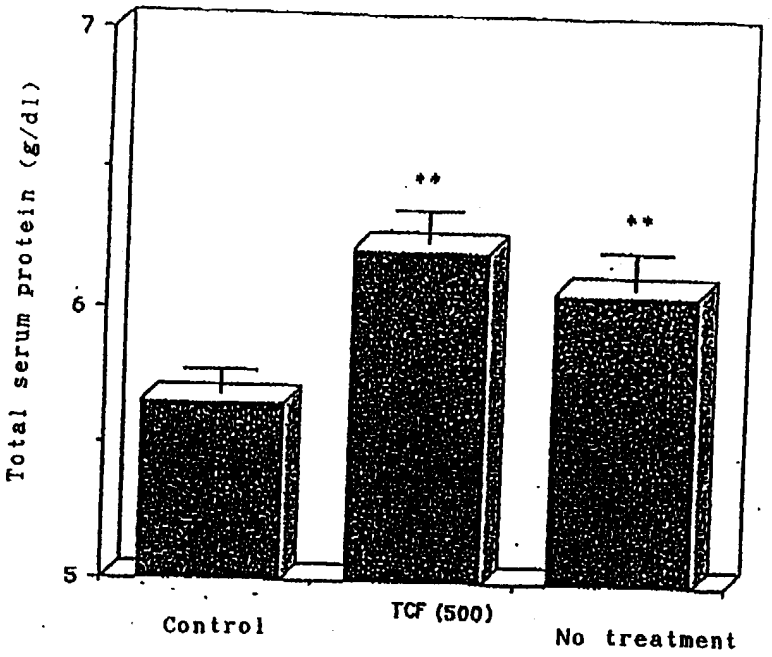


Fig. 22

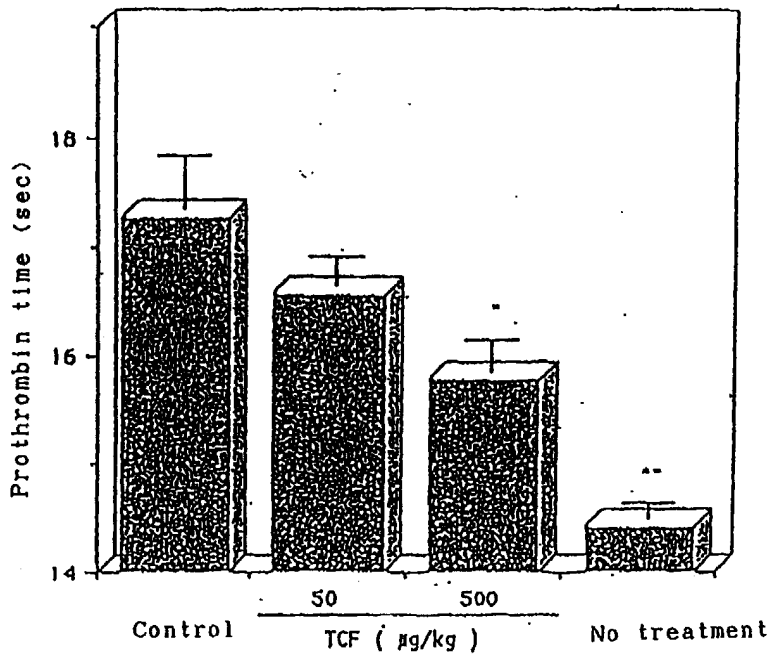

Prothrombin time (sec)
18
16
14
Control
50
500
TCF (μg/kg)
No treatment

Fig. 23

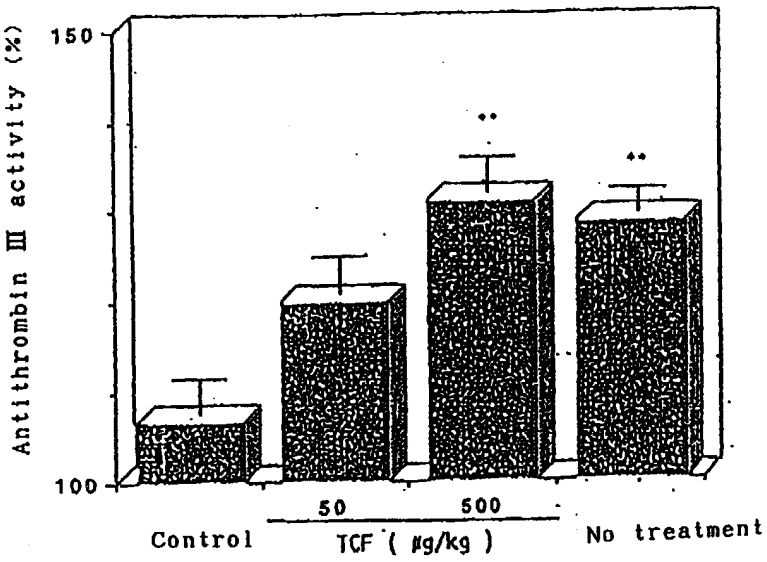

Antithrombin III activity (%)
150
100
**
**
50
500
Control
TCF (μg/kg)
No treatment

Fig. 24